

The effect of a multicomponent intervention on hepatic steatosis is partially mediated by the reduction of intermuscular abdominal adipose tissue in children with overweight or obesity; the EFIGRO project

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Abbreviations

AGReMA; A guideline for reporting mediation analyses.

IMAAT: Intermuscular abdominal adipose tissue

MAFLD: Metabolic-associated fatty liver disease

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

SPSS: Statistical Package for Social Sciences

Abstract

Objective

In adults, there is evidence that the improvement of metabolic associated fatty liver disease (MAFLD) depends on the reduction of myoesteatosis. In children, where the prevalence of MAFLD is alarming, this muscle-liver crosstalk has not been tested. Therefore, we aimed to explore whether the effects of a multicomponent intervention on hepatic fat were mediated by changes on intermuscular abdominal adipose tissue (IMAAAT) in children with overweight/obesity.

Research design and methods

A total of 116 children with overweight/obesity were allocated to a 22-week family-based lifestyle and psychoeducational intervention (control group, N=57), or the same *plus* supervised exercise (exercise group, N=59). Hepatic fat percentage and IMAAT were acquired by magnetic resonance imaging at baseline and at the end of the intervention.

Results

Changes in IMAAT explained the 20.7% of the improvements in hepatic steatosis ($P < 0.05$). Only children who meaningfully reduced their IMAAT (i.e., responders), improved hepatic steatosis at the end of the intervention (within-group analysis; responders: -20%, $P = 0.005$ vs. non-responders: -1.5%, $P = 0.803$). Between-group analysis showed greater reductions in favour to the IMAAT responders compared to the non-responders (18.3% vs. 0.6%, $P = 0.018$) regardless of overall abdominal fat loss.

Conclusions

The reduction of IMAAT has a relevant role over the improvement of hepatic steatosis after a multicomponent intervention in children with overweight/obesity. Indeed, only those children who achieved a meaningful reduction in IMAAT at the end of the intervention reduced their percentage hepatic fat independently of abdominal fat loss. Our findings suggest that abdominal muscle fatty infiltration could be a therapeutic target for the treatment of MAFLD at childhood.

Introduction

Non-alcoholic fatty liver disease (NAFLD, recently named as metabolic-associated fatty liver disease, MAFLD) is currently a leading cause of cirrhosis and hepatocellular carcinoma worldwide (1). Indeed, MAFLD has become the most common chronic liver disorder affecting 25% of the world adult population (1).

In parallel with the childhood obesity epidemic, paediatric MAFLD is affecting to nearly 8% of the general population and about 35% of children with overweight or obesity (2). Children with MAFLD have higher rates of overall, cancer-, liver- and cardiometabolic specific mortality compared to their peers without MAFLD (3). Thus, paediatric MAFLD is a major public health challenge because of its elevated prevalence, associated morbidity and the expected increase in the short- and middle-term (4).

There is evidence of a muscle-liver crosstalk. Thus, sarcopenia is a risk factor for the development and progression of MAFLD in adults (5). In this line, a previous study in adult patients with morbid obesity, shed light on the potential contribution of fatty infiltration in psoas skeletal muscle mass (i.e., myosteatorsis) on the physiopathology of MAFLD (6). In children, the information available examining the link between myosteatorsis and MAFLD is scarce. We have observed that intermuscular abdominal adipose tissue (IMAAT) was associated with the presence and the degree of hepatic steatorsis in pre-adolescent children with overweight/obesity, supporting the potential contribution of IMAAT on the physiopathology of MAFLD (7). Interestingly, Nachit et al. (6) reported that patients who significantly reduced fat infiltration in the psoas after either a dietary intervention or bariatric surgery achieved NASH (Non-Alcoholic

SteatoHepatitis) improvement, suggesting that fat infiltration in the skeletal muscle may be a relevant therapeutic target for patients with MAFLD.

Exercise-based interventions seem to be effective therapeutic approaches for MAFLD prevention and treatment (8). In our study on preadolescent children with overweight/obesity, we compared the effect of a 22-week family-based lifestyle and psychoeducation intervention with the same intervention *plus* supervised exercise, and observed that only those children participating in the group that exercised significantly reduced hepatic fat (nearly 20%) (9). Noteworthy, reductions in hepatic fat were independent of body mass loss as well as of changes on total and abdominal adiposity. These findings seem to support the role of myosteatorosis as mediator of exercise on hepatic fat reduction and as therapeutic target of MAFLD; yet, this hypothesis needs still to be tested in children where obesity and MAFLD prevalence are alarming.

Therefore, the aim of this study was to explore whether the effects of a multicomponent intervention including exercise on hepatic fat were mediated by changes on IMAAT.

Research design and methods

Study design and study subjects

This study is under the umbrella of the EFIGRO project (NCT02258126), a clinical trial designed to compare the effect of a family-based lifestyle and psychoeducational intervention (control group) with the same intervention *plus* supervised exercise (exercise group) on hepatic fat percentage in preadolescent children with overweight/obesity (9). Out of 125 children assessed for eligibility, a total of 116 (aged 8 to 12 years) were allocated to control (N = 57) or exercise (N = 59) groups. Detailed information about inclusion/exclusion criteria, methodology, design of the intervention program, etc., can be found elsewhere (9,10). In brief, children were eligible if they

presented overweight or obesity defined by the World Obesity Federation (11), had 8-12 years of age, did not present any medical condition that limited their activity, and did not have diabetes or any other endocrine disorder.

All parents or legal guardians and their children gave their consent to participate in the study. The study protocol was approved by the Euskadi Clinical Research Ethics Committee, following the ethical guidelines of the Declaration of Helsinki (revised version 2013).

Intervention arms

Children were allocated to the control or exercise groups after baseline measurements. At baseline, there were no significant difference between the two groups in body mass index, age and puberty stage (9). The control group received a family-based lifestyle and psychoeducational intervention (2 sessions/month, 45 min/session) which consists on a total of 11 sessions focused on the following contents: i) dietary habits, ii) physical activity, iii) sleep hygiene, iv) communication skills, and v) feelings and emotions. Sessions were given separately for parents and children (10).

The exercise group received the same family-based lifestyle intervention *plus* supervised exercise training (3 sessions/week, 90 min/session) during 22-week. Briefly, the exercise intervention consists on high intensity (>76% heart rate peak) aerobic and resistance training. To encourage children to spend as much time as possible in high intensity, we used motivation strategies through games. Children were monitored during the sessions by heart rate monitors (Polar RS300X).

Hepatic fat and intermuscular abdominal fat fraction assessments

Hepatic fat percentage and IMAAT were assessed by magnetic resonance imaging (Magnetom Avanto, 1.5T, Siemens Healthcare, Germany). Images were acquired with

breath holding during expiration. Sagittal, coronal, and transverse abdominal localizers (from the diaphragm to the symphysis pubis) were used to determine the accurate location of each image with respect to the vertebral discs. For hepatic fat quantification, detailed information can be found elsewhere (10). Briefly, two different 3D gradient-echo sequences were used in breath-hold, running Siemens Medical System software v.syngo.MR B17A following the manufacturer instructions (12). For IMAAT, fat segmentation was calculated at three axial slices (L2-L3, L3, and L4-L5) and the average was used for analyses. Data processing steps of the IMAAT images can be found in **Fig. 1**. Briefly, T1-weighted images were collected. Next, a 2-point Dixon gradient-echo pulse sequence was used in order to separate the tissue water signal from the lipid signal, resulting on four different images: water-only, fat-only, in-phase, and out-of-phase images. Additionally, two images were calculated: fat fraction or parametric image and $2F - W$ image (where F is the image of the fat, and W is the image of the water). Then, Otsu thresholding algorithm was used for analyses (13). Lastly, a non-supervised clustering (K-means) method was applied to the abdominal visceral compartment using the fat fraction image (14,15). The medical imaging group performed all the analyses and were blinded to the participants' group.

Other potential contributors: sensitivity analyses

For exploratory purposes, we examined the mediating role of cardiometabolic and diabetes risk factors over the hepatic fat reduction at the end of the intervention. Serum triglycerides (mmol/L), high- and low-density lipoprotein (mmol/L), insulin (IU/mL), fasting glucose (mmol/L), homeostatic model assessment, and gamma-glutamyl transferase (units/L) were measured from morning fasting blood samples collected at the hospital.

Overall abdominal adiposity was measured by Dual-energy X-ray absorptiometry (Hologic QDR, 4500W).

Statistics

All data are presented as mean and standard deviation unless specified otherwise. We assessed normality of the variables used in the analyses by visual (Q-Q plots) and statistical (Kolmogorov-Smirnov) tests, and therefore, parametric tests were used. To test whether the effects of the intervention on changes on hepatic fat were mediated by changes on IMAAT, mediation analyses were performed after adjusting for age, sex, and hepatic fat at baseline. The unstandardized (B) and standardized beta regression coefficients are presented for the following equations: i) regressed the mediator (i.e., change in IMAAT) on the independent variable (i.e., group), ii) regressed the dependent variable (i.e., change in hepatic fat) on the independent variable (group), iii) regressed the dependent variable on both the mediator and the independent variable. Indirect and total effect were also presented, and thus, the percentage of the total effect was computed in order to explain how much of the total effect was explained by the mediation. As sensitivity analyses, we additionally explored whether other cardiometabolic and diabetes risk factors (i.e., triglycerides, high-density lipoprotein, low-density lipoprotein, insulin, glucose, homeostatic model assessment, and gamma-glutamyl transferase) could further contribute on the hepatic fat reduction. The mediation analyses are in line with the AGReMA statement (A Guideline for Reporting Mediation Analyses, <https://agrema-statement.org/>; Supplemental Content 1) (16). Further, to explore differences in changes on percentage hepatic fat between participants (irrespective of their assigned group) who experienced a meaningful change (i.e., responders in both groups, Cohen's $d \geq 0.2$, N= 59) or not (i.e., non-responders in both groups, Cohen's $d < 0.2$, N = 39) from baseline to post-intervention in IMAAT, paired

t-student test (within-group analyses) and one-way analyses of covariance (between-group analysis) were applied.

The main analyses are presented following the *per protocol* principle which comprise all participants who finished the intervention and attended at least 50% of the family-based lifestyle and psychoeducational sessions (in the exercise group, no minimum attendance of exercise sessions was required). As sensitivity analyses, we performed the analyses using a modified *per protocol* principle which additionally comprises a minimum of 50% attendance of the exercise sessions. The *intention-to-treat* principle was based on missing values at post-intervention obtained by multiple imputation.

Imputation was performed using the pre- and post-intervention values, age, sex, and intervention group. *Intention-to-treat* analyses are presented in Supplemental content.

All the analyses were performed using the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc. Chicago, IL) and its PROCESS macro.

Mediation analyses were applied with a resample procedure of 10000 bootstrap samples. The difference was considered as significant when the indirect effect significantly differed from zero. For the rest of analyses, the significance level was set as $\alpha < 0.05$. Figures were created with R software (v. 4.0.3, <https://cran.r-project.org/>).

Results

Descriptive characteristics of the study subjects

Fig. 2 shows the participant flow diagram of this study. Out of 116 children initially allocated, 98 participants (10.5 ± 1.1 years, 56% girls) successfully completed the trial attending at least 50% of the family-based lifestyle and psychoeducational intervention with no minimum attendance of exercise sessions for the exercise group, i.e., *per protocol* analysis. There was no significant difference in terms of attendance at the

lifestyle- and psychoeducation program sessions between the two groups (control *vs.* exercise), either for the parents/caregivers (86.4±12.9% *vs.* 80.6±15.3%; P=0.334) or the children (87.2±12.0% *vs.* 82.5±14.6%; P=0.496). The mean attendance rate of the children to the exercise program was 72.0±16.1% sessions. No adverse events were observed as a consequence of the family-based lifestyle and psychoeducational intervention, whilst two participants showed exercise-related adverse events including knee and ankle pain.

Baseline characteristics of the study participants grouped by intervention groups (control *vs.* exercise) and by interindividual variability (non-responders *vs.* responders) can be found in **Table 1** and Supplemental Table S1, respectively.

Linking abdominal muscle fat and hepatic fat: mediation and responders' analyses

Fig. 3 shows that the reduction of IMAAT explained 20.7% of the effect of the intervention on percentage hepatic fat at the end of the intervention (indirect effect $\beta = -0.099$, 95% CI = -0.210, -0.010). *Intention-to-treat* analysis showed similar findings (Supplemental content Fig. S1). We additionally explored whether other cardiometabolic and diabetes risk factors (i.e., triglycerides, high-density lipoprotein, low-density lipoprotein, insulin, glucose, homeostatic model assessment, and gamma-glutamyl transferase) could further contribute on the hepatic fat reduction. The findings showed that these factors did not contribute of the effect of the intervention on percentage hepatic fat at the end of the intervention. The changes in hepatic fat percentage at the end of the intervention grouped in non-responders and responders for IMAAT can be found in **Fig. 4**. In non-responders for IMAAT (i.e., children whose reduction in IMAAT was <0.2 Cohen's d), there was no significant reduction of percentage hepatic fat (change = -1.5%, P = 0.803). In contrast, percentage hepatic fat

was significantly reduced in children categorized as responders for IMAAT (change on percentage hepatic fat = -20%, $P = 0.005$). Between-group analysis showed that the reduction on percentage hepatic fat was significantly greater in responders for IMAAT than in non-responders (-0.6% vs. -18.3%; age, sex and baseline hepatic fat adjusted $P = 0.018$). Interestingly, further adjustment with changes in overall abdominal adiposity did not substantially change the result (0.4% vs. -18.9%, $P = 0.021$). Similar findings were observed in the *intention-to-treat* analysis (Supplemental content Fig. S2). As sensitivity analyses, we repeated the analyses including only children with MAFLD (i.e., having > 5% hepatic fat, $N=22$ in the group of responders and $N=18$ in the group of non-responders) and we observed similar results (-9.1% vs. -31.1%, $P = 0.029$).

Finally, we explored whether the effects of exercise on IMAAT and hepatic fat depended on the number of exercise sessions attended. We observed that there was no significant correlation between the number of exercise sessions attended and the changes on IMAAT and hepatic fat in the exercise group (all $p > 0.05$). As sensitivity analyses, using a modified *per protocol* principle, we additionally included a minimum of 50% of attendance to the exercise sessions (four participants were excluded for not meeting this *per protocol* criteria; n total = 94 participants). The results showed similar findings to the original *per protocol* principle applied (e.g., changes in IMAAT explained the 22.6% of the improvements in hepatic fat).

Conclusions

The present study shows the mediating role of the reduction of IMAAT over the improvement of hepatic steatosis after a multicomponent intervention in children with overweight/obesity. Indeed, only those children who achieved a meaningful reduction in IMAAT at the end of the intervention reduced their percentage hepatic fat, and this

change was independent of abdominal fat loss. Our data thus suggest that abdominal muscle fatty infiltration could be a therapeutic target of hepatic steatosis treatments in children with overweight/obesity.

In the recent years, there is growing interest on the physiopathological mechanisms linking the muscle-liver axis. Although most of the literature published in this topic is focused on thigh, psoas or calf muscle fat, there is an emerging body of evidence focused on abdominal muscle fat (7,17). Nowadays the evidence is still very limited and focused on animal models (18) or adult population (6,19,20). Our work is the first study assessing the role of abdominal muscle fat over hepatic fat after a multicomponent intervention in children with overweight/obesity. In agreement with our findings, Nachit et al. (6) observed that NASH improvement was associated with a significant decrease of psoas muscle fat content in patients with morbid obesity after two different interventions (i.e., 12-month of dietary or bariatric surgery). In this line, at cross-sectional level, previous authors have shown a relationship between muscle fat content (measured at thigh, psoas or multifidus muscle level) and fatty liver disease in humans and animals models (6,19,20). Therefore, it seems that there is a generalized muscle-liver relationship, independently of the IMAAT area located (21). Further, our previous results in this cohort of children provide new insights on the abdominal skeletal muscle-liver association; likewise, children with MAFLD presented higher IMAAT compared to those without (7). Taking together our findings with the previous literature, it might be expected that IMAAT reduction is a marker of overall improved myosteatorsis.

We previously reported that a multicomponent intervention program, which comprises exercise training in addition to a family-based lifestyle and psychoeducational intervention, is more effective reducing percentage hepatic fat (-20%) than the lifestyle intervention alone (0%) (9). In the current study, we have observed that the effects of

the intervention are partially mediated by the reduction of IMAAT. Herein, our results suggest that the addition of exercise training to the intervention program could be a key factor for achieving abdominal muscle fat reduction and, then, to reduce in hepatic steatosis in children with overweight/obesity. A previous study in adolescents with overweight/obesity showed that regular exercise was effective in reducing intermuscular adipose tissue at the *fascia lata* surrounding skeletal muscle, and the adipose tissue area between muscle bundles, highlighting the usefulness of regular exercise as an important therapeutic strategy to reduce myosteatorosis (22). In this regard, two previous systematic reviews observed that exercise and weight loss may act directly decreasing IMAAT, and improving several cardiometabolic risk factors such as insulin sensitivity and inflammation, muscle strength and quality (23,24). Our results, together with the reported anti-inflammatory effects of exercise training (23) and the effectiveness of physical activity on skeletal muscle metabolism (24) suggest that exercise (i.e., aerobic training, muscular training, and/or its combination) produces specific effects on abdominal muscle mass reducing IMAAT independently of the effects on other fat depots. Indeed, skeletal muscle seems to be the major location for whole body insulin-stimulated glucose disposal (25,26). In adults with obesity and type-2 diabetes, the increment of IMAAT was associated with insulin resistance (27,28). Overall, the causative effect of muscle and liver fat can be inferred from the present results and, therefore, we could state that myosteatorosis contributes to MAFLD progression by the presence of peripheral insulin resistance and/or perturbation of the muscle metabolism and secretome associated with muscle fat (6,29–31). As example, in a preclinical model with mice, Nachit et al. (18) observed that severe myosteatorosis was a consistent, specific, and early marker of NASH in MAFLD. Therefore, our findings in children together with those reported in adolescents (22) and adults (6), reinforce the importance

of reducing skeletal muscle fat infiltration, with special emphasis on IMAAT, to improve liver health and insulin sensitivity.

Limitations and strengths

This study presents limitations: the study design was not entirely strictly randomized (i.e., 11 children/families were not available to attend the exercise sessions and, thus, were allocated to the control group); yet, the participants allocated in both groups were comparable at baseline and adjustments for potential baseline differences between control and exercise groups were performed in analyses. Moreover, we have repeated all the analyses excluding those children/families that were not randomized, and the findings obtained were quite similar to those presented in the main manuscript. On the other hand, the strengths of this study were: i) the novelty of the study in children with overweight/obesity, ii) the relatively large sample size included in the study with abdominal muscle and hepatic fat measurements using magnetic resonance imaging, iii) the medical imaging group who analysed the main outcomes of this study (i.e., images from magnetic resonance imaging) were entirely blinded to the intervention groups, and iv) the performance of family-based lifestyle and psychoeducational intervention plus supervised exercise training following pediatric childhood obesity guidelines (32).

Conclusions

In summary, abdominal muscle fat has a relevant role over the reduction on hepatic fat after a multicomponent intervention in children with overweight/obesity, regardless of abdominal fat loss. These data indicate that muscle fatty infiltration could be a therapeutic target already at childhood. Future studies are needed to corroborate or contrast our findings.

Acknowledgments

Authors' contributions

CC-S.: conception of the study, analysed the data, and drafted the manuscript; FI, RC, AV, BR-V, MM, and MO: acquisition, analysis, and interpretation of the data, and critically review the manuscript. FBO and JRR: interpretation of the data, and critically review the manuscript. IL: conception of the study, analysed and interpretation of the data, and critically review the manuscript. The authors declare they have seen and approved the final version of the manuscript. CC-S and IL are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Table 1. Baseline characteristics of the children participating in the study across intervention groups.

	Control group		Exercise group	
	N	Mean, SD	N	Mean, SD
Age (years)	57	10.6, 1.1	59	10.5, 1.0
Body mass index (kg/m ²)	57	25.2, 2.8	59	25.8, 3.7
Hepatic fat (%)	56	5.3, 2.9	59	6.0, 4.8
IMAAT (%)	57	7.0, 1.1	59	7.2, 0.9
	N	n, %	N	n, %
Girls	57	30, 52.6	59	32, 54.2
Tanner stage				
Telarche or gonadarche	55		53	
I		20, 36.4		22, 41.5
II		20, 36.4		12, 22.6
III		12, 21.9		11, 20.8
IV-V		3, 5.5		8, 15.1
Pubarche	55		53	
I		20, 36.4		17, 32.1
II		22, 40.0		20, 37.7
III		7, 12.7		10, 18.9
IV-V		6, 10.9		6, 11.3
MAFLD presence	56	23, 41.1	59	28, 47.5

IMAAT: Intermuscular abdominal adipose tissue. MAFLD: Metabolic-associated fatty liver disease.

Control group consisted on two family-based lifestyle and psychoeducational sessions/month. Exercise group consisted on two family-based lifestyle and psychoeducational sessions/month *plus* 3 sessions/week of supervised high-intensity exercise. MAFLD presence was defined as having > 5% hepatic fat.

Figure legends

Figure 1. Intermuscular abdominal adipose tissue processing steps.

F: Fat. IMAAT: Intermuscular abdominal fat fraction. SIF: Fat-only. SIW: Water-only.

Figure 2. Flow diagram of the data collection and analysis of the study.

ITT: Intention-to-treat. MRI: Magnetic Resonance Imaging. Control group consisted on two family-based lifestyle and psychoeducational sessions/month. Exercise group focused on two family-based lifestyle and psychoeducational sessions/month plus 3 sessions/week of supervised high-intensity exercise.

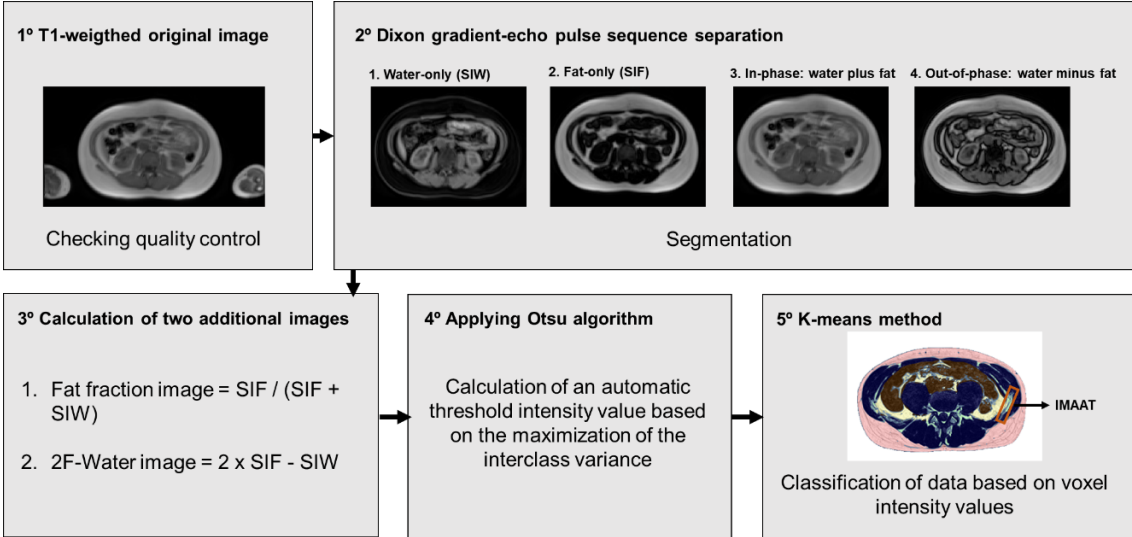
Figure 3. Mediation model to determine whether changes in IMAAT mediated changes in hepatic fat percentage. *Per protocol analysis*.

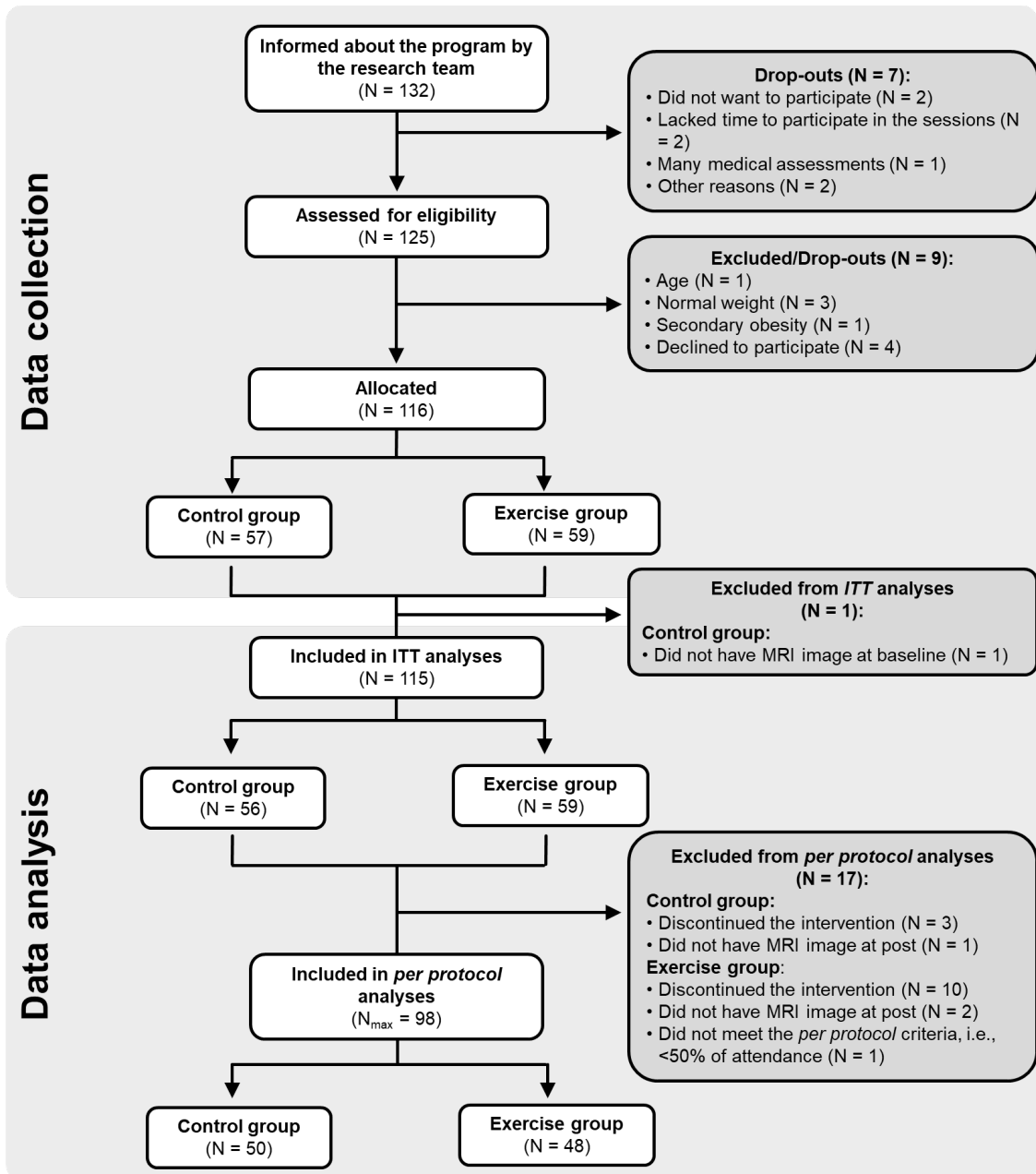
Data presented following *per protocol* analysis, i.e., attending at least 50% of the educational program sessions (in the exercise group, no minimum attendance of exercise sessions was required). Analyses were adjusted for baseline values, age, and sex. Delta (Δ) expresses the outcome at post-intervention with respect to baseline.

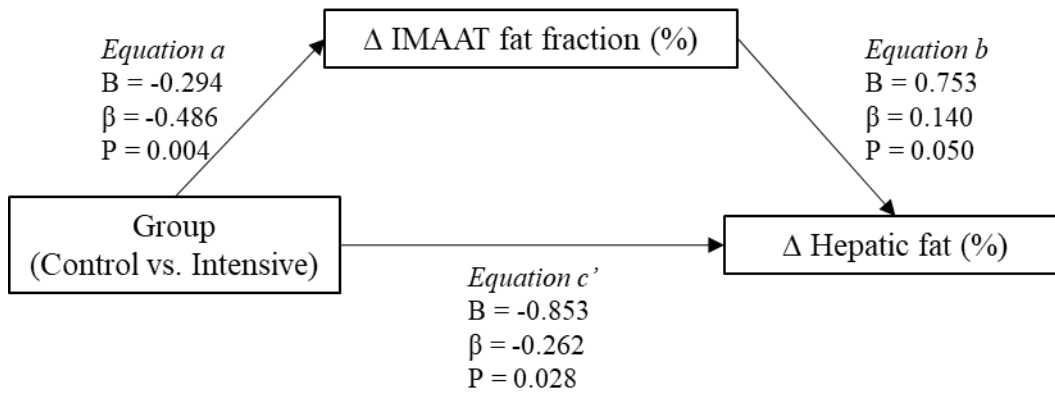
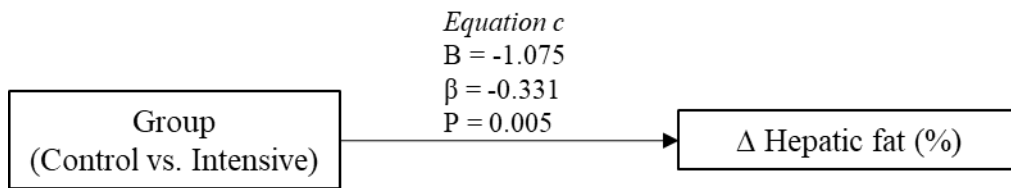
β : Standardized beta coefficient. CI: Confidence Interval. IMAAT: Intermuscular abdominal adipose tissue.

Figure 4. Changes in hepatic fat (%) in the group of children who did not experience a meaningful intermuscular abdominal adipose tissue (IMAAT) reduction (non-responders) and in the group of children who experience a meaningful IMAAT reduction (responders) after the intervention. *Per protocol analysis*.

Panel A shows boxplots of the hepatic fat percentage by IMAAT non-responders (coloured in grey colour) and responders (colour in pink colour) at baseline (pre) and after 22-week intervention. The ends of the boxes in the boxplots are located at the first and third quartiles, with the black line in the middle illustrating the median. Whiskers extend to the upper and lower adjacent values, the location of the furthest point within a distance of 1.5 interquartile ranges from the first and third quartiles. The parallel line plot contains 1 vertical line for each participant which extends from their baseline to their 22-week value. Descending lines indicate a reduction in hepatic fat. Participants were classified as responders when they reduce the IMAAT fat fraction after the intervention (Cohen's $d \geq 0.2$), whilst non-responders were categorized for those participants who did not experience a reduction (Cohen's $d < 0.2$). Pre-test values are placed in ascending order for the non-responders' group (in black colour) and descending order for the responders' group (in pink colour). Panel B shows boxplots of the change in hepatic fat percentage. Changes were calculated as post- minus pre-intervention values. Analyses were adjusted for baseline values, age, and sex. Data analyses were conducted under *per protocol* principle, i.e., attending at least 50% of the educational program sessions (in the exercise group, no minimum attendance of exercise sessions was required).

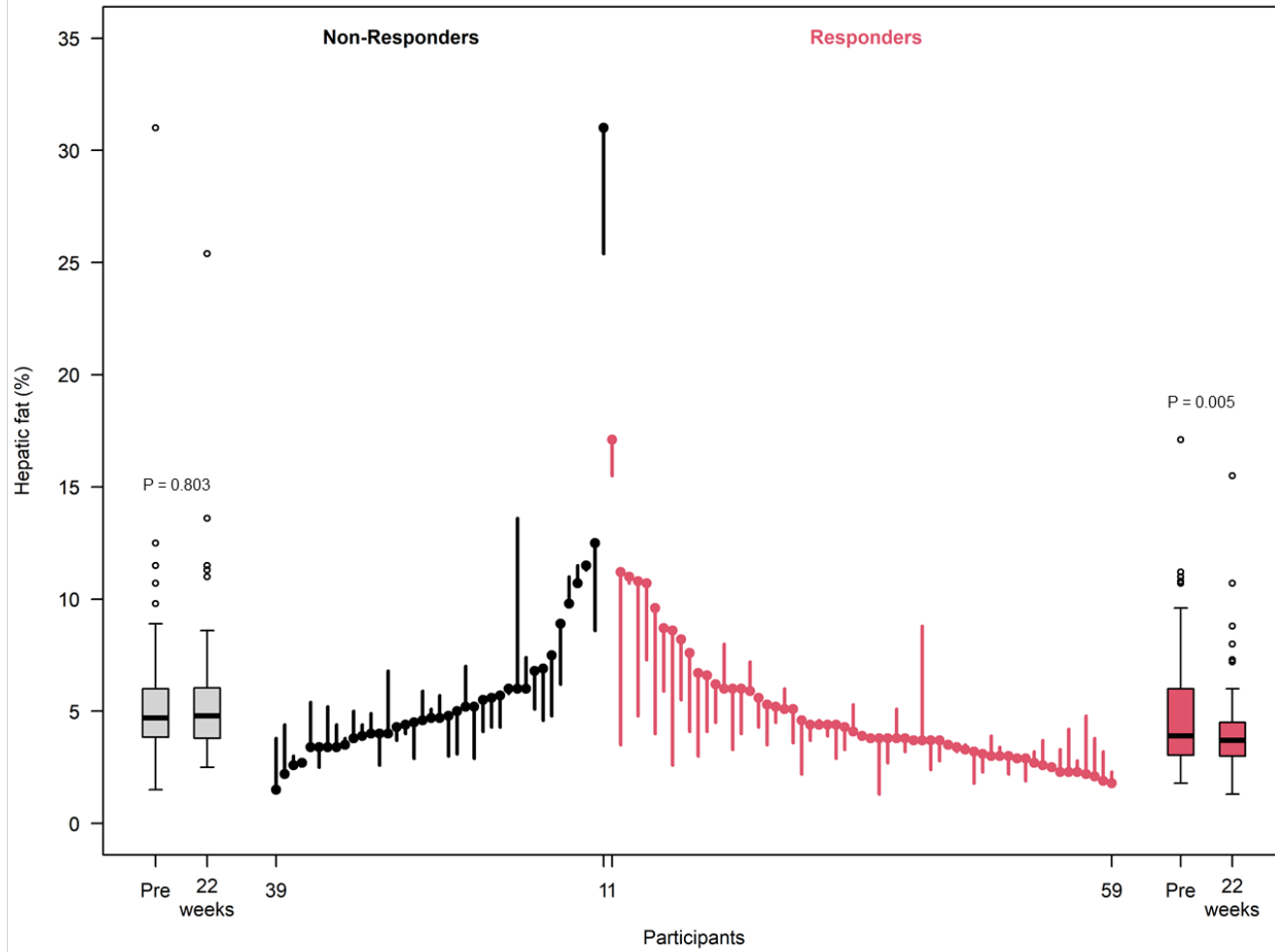






Indirect effect
 $B (CI) = -0.222 (-0.482, -0.021)$
 $\beta (CI) = -0.099 (-0.210, -0.010)$
Percentage of the total effect = 20.7%

A)



B)

